

Autotrophic and heterotrophic soil respiration responds asymmetrically to drought in a subtropical forest in the Southeast China

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ABSTRACT

Both increasing frequency of drought and drought duration are expected for many terrestrial ecosystems under on-going climate change. However, our understanding of the drought effect on soil respiration (R_s), which comprises the second largest carbon (C) flux of the global C cycle, remains limited. To explore the effects of reduced precipitation on R_s and its components, we conducted an experiment of throughfall rainfall exclusion during two consecutive growing seasons in a subtropical forest in the Southeast China. Following throughfall exclusion R_s declined rapidly, and did not recover until three to four months following rewetting, in both 2014 and 2015. During the experiment, throughfall exclusion significantly reduced autotrophic soil respiration (R_a); however, heterotrophic soil respiration (R_h) was unaffected, resulting in a reduced contribution (R_a/R_s) from $33 \pm 1\%$ for the control to $16 \pm 3\%$ under throughfall exclusion. Experimental drought significantly reduced soil microbial C and fine root biomass, and subsequent to rewetting, soil microbial C recovered quickly, but fine root biomass relapsed slowly. Our results suggested that prolonged drought decreases R_s through modifications in soil microbial activities and fine root metabolic capacity, which are induced by reduced soil water availability. Moreover, our results imply that drought-induced reductions in R_s originate primarily from R_a . Our results highlight the need to account for asymmetric responses to drought between R_a and R_h when predicting the reaction of the ecosystem C balance in response to future drought events.

1. Introduction

Soil respiration (R_s) constitutes the second-largest terrestrial carbon (C) flux (Bond-Lamberty and Thomson, 2010), releasing approximately 58 Pg CO₂ annually from terrestrial ecosystems, which is 10-fold that of anthropogenic combustion emissions (Hanson et al., 2000; Schlesinger and Andrews, 2000). Therefore, even subtle changes in R_s can have a significant impact on the global C cycle. Both soil temperature and soil moisture are critical environmental determinants for R_s (Laganière et al., 2012; Lellei-Kovács et al., 2016). Previous studies have indicated that global warming accelerates R_s (Lu et al., 2013; Noh et al., 2015; Xu et al., 2015). However, the impacts of extreme droughts, whose intensity and frequency are anticipated to increase during the 21st century (IPCC, 2013), on R_s remain uncertain. Previous precipitation manipulation experiments have revealed that the effects of reduced precipitation on R_s are highly variable, and their effects tend to be

contingent on soil moisture conditions (Borken et al., 2006; Sotta et al., 2007; Cleveland et al., 2010; Doughty et al., 2015). In addition, the responses of R_s to drought may differ between growing and non-growing seasons (Suseela and Dukes, 2013; Zhang et al., 2015).

Soil moisture directly influences R_s through physiological processes (Manzoni et al., 2012; Schindlbacher et al., 2012; Li et al., 2013), and indirectly by diffusion of oxygen through the substrate (Cleveland et al., 2010; Van Straaten et al., 2011). Predicting the response of R_s to altered soil water availability is inherently difficult, as R_s includes both heterotrophic (R_h) and autotrophic (R_a) components (Kuzakov, 2006; Hinko-Najera et al., 2015). The R_h depends on microbial activity and substrate availability (Scott-Denton et al., 2006; Hinko-Najera et al., 2015), while R_a is regulated by the allocation of recently assimilated C (Hanson et al., 2000; Kuzakov, 2010; Doughty et al., 2015). Recent studies have revealed that R_a and R_h respond differently under drought conditions (Borken et al., 2006; Sanaullah et al., 2012; Kopittke et al.,

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Table 1Soil properties of different soil layer in the evergreen broadleaved forest (mean \pm s.e.m., $n = 3$).

Soil depth (cm)	Bulk density (g·cm ⁻³)	SOC (g·kg ⁻¹)	TN (g·kg ⁻¹)	MBC (mg·kg ⁻¹)	pH (H ₂ O)	Soil texture (%) ^a		
						Sand	Silt	Clay
0–10	1.06 \pm 0.02	55.4 \pm 0.8	3.2 \pm 0.1	665.0 \pm 34.0	4.5 \pm 0.1	68.8	20.0	11.2
10–20	1.10 \pm 0.02	32.9 \pm 1.7	2.0 \pm 0.2	377.3 \pm 14.1	4.8 \pm 0.1	63.2	25.8	11.0
20–40	1.18 \pm 0.01	19.6 \pm 0.8	1.3 \pm 0.1	161.6 \pm 14.3	4.9 \pm 0.2	59.4	16.7	23.9
40–60	1.27 \pm 0.02	12.6 \pm 1.1	0.8 \pm 0.1	168.1 \pm 19.1	5.1 \pm 0.1	–	–	–

^a Bu et al. (2012).

2014); however, it remains unclear whether R_a or R_h is more sensitive to droughts.

In addition, how R_s and its components respond to subsequent rewetting also remains uncertain. Rewetting induced R_s bursts, referred to as the “Birch effect” (Birch, 1958), have been observed in a temperate forest soil (Borken et al., 2006). However, these R_s bursts appear to be intimately dependent on soil properties, vegetation type, and the length and intensity of the drying-wetting cycle (Muhr et al., 2011; Manzoni et al., 2012; Hagedorn and Joos, 2014). These findings indicate that different magnitudes and directions of R_s in response to rewetting occur, making predictions of soil C balances under increasing drought conditions rather difficult (Hagedorn and Joos, 2014).

The subtropical region of China is characterized as an extensive C reserve with high rates of C fluxes that are sustained by abundant light, heat, and water resources (Yu et al., 2014). Due to low atmospheric conditions that are controlled by a West Pacific Subtropical High-Pressure System, in combination with a prevailing descending airflow, this region is often impacted by high temperatures and severe drought stress during the summer (Mi et al., 2009). Moreover, this region is expected to experience an increasing probability of drought intensity and frequency in the 21st century (Dai, 2011). More frequent or prolonged droughts can significantly reduce soil water availability, and in turn, could strongly impact C cycles, plant productivity, and biodiversity (Wessel et al., 2004; Beier et al., 2012).

Here, we employed a rainfall throughfall exclusion (TFE) experiment to simulate the effects of growing season drought on R_s in an evergreen broadleaved forest in subtropical China. Our goal was to determine the impacts of reduced soil water availability on R_s . Our drought experiment included two periods, one in the summer of 2014, and the other in the spring of 2015. We hypothesized that water reduction would decrease R_s , and the reduced R_s might primarily result from R_h , as low soil water availability limits R_h more than R_a components (Borken et al., 2006). We expected a rapid increase in R_s with rewetting following a spring drought due to abundant summer rainfall, but a slow R_s recovery following a summer drought due to limited autumn rainfall. We quantified soil temperature, soil moisture, soil microbial biomass C (MBC), and fine root biomass (FRB) to better understand the links between the changes in R_s and its components that were induced by droughts.

2. Materials and methods

2.1. Site description

Our study was conducted in the Wuyi Mountain National Nature Reserve (27°33′–27°54′N, 117°27′–117°51′E), with an area of 56,527 ha, which is located in the Southeast China. The typical forest in this region is evergreen broadleaved forest (Yu et al., 2014). The climate is mid-subtropics with a mean annual temperature of 15.2 °C and mean annual precipitation of 2764 mm from 2006 to 2016. Approximately 70% of the precipitation fell in the growing season, from March to August (data from a meteorological station that was 500 m away from the site).

The experiment was conducted within a 15 ha evergreen broadleaved forest (tree ages ranged from 60 to 70 years) at an altitude

ranging from 620 m to 674 m a.s.l., and slopes ranging from 30° to 32°. This area was dominated by *Castanopsis eyrie* Tutch, *Cyclobalanopsis myrsinifolia* Oersted and *Castanopsis carlesii* Hay, accounting for 78% of the overstory stand basal area. Based on the measurements of three plots (30 m \times 30 m for each), the mean of tree height and diameter at breast height were 11.6 \pm 1.6 m and 15.5 \pm 2.3 cm, respectively. The stand had 90% of tree canopy cover, 1656 trees ha⁻¹ of stem density, and 30.9 m² ha⁻¹ of basal area. The shrub layer vegetation consisted primarily of *Rapanea neriifolia*, *Engelhardia fenzelii* and *Indocalamus tessellates*. The bulk density, organic C, and total nitrogen concentration of the soil in the 0–10 cm mineral soil layer were 1.06 \pm 0.02 g cm⁻³, 55.4 \pm 0.8 g kg⁻¹, and 3.2 \pm 0.1 g kg⁻¹, respectively. Soil properties in other soil layers are presented in Table 1. The litter layer thickness ranged from 1 to 3 cm, and the annual litterfall was 3.8 \pm 0.2 t ha⁻¹ yr⁻¹ between 2003 and 2012.

2.2. Experimental design

In the winter of 2013, three experimental plots (30 m \times 30 m) were randomly established within the 15 ha evergreen broadleaved forest with similar topography. These plots were approximately 200 m apart. For each of the three plots, four 3 m \times 3 m subplots were established with 10 m buffer zones between them. No tree trunks existed in any control and treatment subplots. Two of the four subplots received throughfall exclusion (TFE) treatment, whereas the other two served as the control treatment. Trenches were dug, and all roots were cut off in one of the TFE subplots and one of the control subplots to estimate the autotrophic and heterotrophic components of soil respiration (Schindlbacher et al., 2009; Noh et al., 2015). To minimize the transient response caused by decomposition of dead roots, the trenching treatment was conducted six months before soil efflux measurements began in the trenched subplots (Noh et al., 2017). Trenches were dug in an area of 1 m \times 1 m, and were excavated to a depth of 60 cm (below which few roots existed) (Bu et al., 2012), or to bedrock, when the soil depth was < 60 cm. After lining the trench with a double-layer polyethylene film, the soil was backfilled. The aboveground components of the understory vegetation in the trenched subplots were carefully removed with minimal soil disturbance, and were kept free of any vegetation by repeated manual removal throughout the entire study period. The perimeter of the drought treatment subplots was also trenched to a depth of 30 cm and lined with double-layer polyethylene film to prevent surface run-off and lateral water movement into the subplots.

We installed portable rain shelters (3 m \times 3 m) that were covered with transparent polyethylene film, and framed using tunnel-shaped galvanized aluminum tube supports with roughly 2.0 m in height. The rain shelters were covered between June 13th and September 12th, 2014, and between March 15th and June 27th, 2015. Throughfall that accumulated on the roofs during both TFE treatment periods was drained into gutters over a distance of about 20 m before it flowed into the ground external to the plots. The sidewalls of the rain shelters were left open, which facilitated air flow to minimize the difference in temperature and humidity between their interiors and exteriors (Sherman et al., 2012). The polyethylene films were removed on

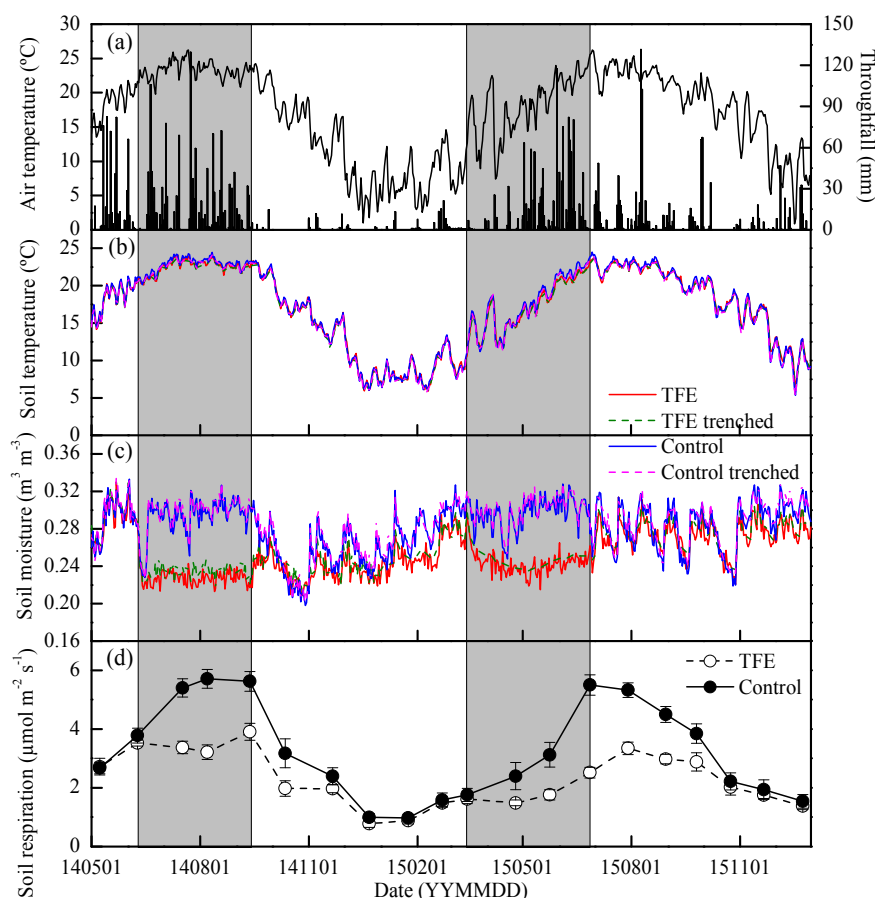


Fig. 1. Seasonal variation in (a) air temperature and throughfall, (b) soil temperature, (c) soil moisture at 5 cm mineral soil depth and (d) soil respiration in the control and throughfall exclusion (TFE) treatments. Gray shaded regions indicate periods when the rain shelter was installed. The experiment was conducted in an evergreen broadleaved forest in the Southeast China, from May, 2014 to December, 2015. Values represent means \pm standard errors ($n = 3$).

September 12th, 2014 and June 27th, 2015, to allow for the rewetting of the soil by precipitation. In the summer of 2014, 1436 mm of throughfall were excluded, in comparison to 1244 mm in the spring of 2015 (Fig. 1a).

Micrometeorological variables were monitored continuously throughout the experimental period. From May 2014 to December 2015, soil temperature at each subplot was measured by S-TMB-M006 temperature sensors ($< \pm 0.2^\circ\text{C}$ accuracy and $< \pm 0.03^\circ\text{C}$ resolution), and soil moisture at each plot was measured by ECH₂O-5 soil moisture probes ($< \pm 2\%$ accuracy and $< \pm 0.1\%$ resolution) at 5 cm mineral soil layers (Onset Computer Corporation, Bourne, MA, USA). The soil moisture and temperature were measured every 20 s and the averaged data were stored on data loggers every 30 min. Also, throughfall and air temperature were recorded using HOBO data logging rain gauges (Onset Computer Corporation, Bourne, MA, USA).

2.3. Soil respiration measurement

At each subplot, two thin-walled polyvinyl chloride respiration collars of 20 cm in diameter and 7.5 cm in height were installed, which were permanently inserted approximately 5 cm into the soil to minimize the potential mechanical disturbance during the measurements. Two collars were installed for each subplot, for a total of 24 collars. We measured R_s using an IRGA infrared gas analyzer (Li-8100, Li-Cor Inc., Lincoln, NE, USA) between May 2014 and December 2015, at monthly intervals, yielding 20 measurements during the study. At each sampling time, measurements were performed between 8:00 and 12:00 h. Each sampling campaign lasted 3–4 h, during which we randomly placed the order of measurements between the control *versus* the drought treatment, and trenched *versus* untrenched subplots, to prevent confounding diurnal trend effects associated with treatments (Schindlbacher et al., 2012; Hagedorn and Joos, 2014). To ensure the measurement stability

of the instrument, R_s was measured twice at each soil collar, where each measurement was 120 s in duration, followed by a 30 s pause between the measurements, during which time the chamber was automatically opened to allow for the replacement of the air in the chamber with ambient atmosphere. The average of the two measurements was recorded as the measured value.

2.4. Fine root biomass and soil microbial biomass C determination

To assess the effects of the drought treatments on fine root biomass (FRB) and soil microbial biomass C (MBC), we sampled the soil prior to, during, and following throughfall exclusion for all drought and control treatment subplots. The FRB was estimated from soil samples collected down to a depth of 10 cm, using a manual corer of 3.8 cm in diameter. Three soil cores were randomly collected from each subplot. Fresh soil samples, adjacent to the fine root core position, were collected at a soil depth of 0–10 cm. Within 48 h of collection, the fine root core and fresh soil samples were transported to the laboratory, where they were stored for no more than 1 week at 4°C prior to FRB and MBC determinations. Each fine root core sample was soaked in water overnight, poured into trays, rinsed, and hand sorted to separate living fine roots ($< 2\text{ mm}$) from the soil. The fine root diameter was determined by using Vernier caliper and the coarse fraction and dead roots were determined via morphology and colour (Brassard et al., 2013; Liu et al., 2016; Feng et al., 2018). Sorted fine roots ($< 2\text{ mm}$) were then oven-dried at 60°C to a constant mass.

The samples for MBC were sieved using a 2 mm mesh, and the MBC was determined using the chloroform (CHCl_3) fumigation-extraction (FE) method (Vance et al., 1987). For each sample, 10 g of dry-weight equivalent soil samples were fumigated with CHCl_3 for 24 h in a vacuum, after which the fumigated and unfumigated samples were extracted with 50 ml 0.5 M K_2SO_4 solutions. The K_2SO_4 extracts were

shaken for 30 min, filtered through 0.45 µm filters, and stored at 4 °C until the analysis of extractable organic C was complete. The quantities of MBC were determined as the difference between extractable organic C in the fumigated and unfumigated samples, using a conversion factor of extractable C ($k_{EC} = 0.45$) (Joergensen, 1996).

2.5. Statistical analyses

We calculated autotrophic soil respiration (R_a) by subtracting R_h in the trenched subplots from R_s measured in the untrenched subplots, which was measured in the corresponding trenched subplots (Hinko-Najera et al., 2015).

$$R_a = R_s - R_h \quad (1)$$

The relative contribution of R_a to R_s was calculated as R_a divided by R_s .

$$RC = \frac{R_a}{R_s} \times 100\% \quad (2)$$

Daily R_s in untrenched and trenched subplots was calculated by linear interpolation between two sequential measurements (Schindlbacher et al., 2012). The cumulative R_s (t C ha⁻¹) in untrenched and trenched subplots was estimated by summing the daily R_s over the respective treatment period (Laganière et al., 2012).

$$\text{cumulative } R_s = \sum_{\text{daily}} R_s \cdot (3600 \cdot 24) \cdot (12/10^{12}) \cdot 10\,000 \quad (3)$$

where multiplied by (3600 times 24) to convert the time, from seconds to days, and by (12/10¹²) to convert C, from micromoles to tons, and by 10 000 to convert area, from square meter to hectare.

For each individual treatment, temperature sensitivity (Q_{10}) was calculated based on the monthly measurements of R_s and soil temperature. The Q_{10} values were calculated according to the following function (Lloyd and Taylor, 1994):

$$R_s = \alpha \times e^{\beta T} \quad (4)$$

$$Q_{10} = e^{10\beta} \quad (5)$$

where T is measured soil temperature at the depth of 5 cm, and α and β are regression coefficients.

Because our measurements of R_s , soil moisture, and soil temperature were temporally autocorrelated with each soil collar, we used repeated measures analysis of variance (ANOVA) to test the effects of treatment and measurement time. We used the Greenhouse-Geisser adjustment when the assumption of sphericity was not met (Gimbel et al., 2015). The assumptions of normality and homogeneous variances were examined by the Shapiro-Wilk's test, and Levene's test, respectively. The corresponding variables were transformed using a base 10 logarithm to better meet the assumptions of normality and homogeneity. The treatment effect on the cumulative R_s , Q_{10} , FRB, and MBC was tested by

one-way ANOVA. All statistical analyses with a significance level of 0.05 were applied using SPSS version 17.0 for Windows (SPSS, Chicago, Illinois, USA), and figures were generated using Origin 8.5 (Origin 8.5, Origin-Lab).

3. Results

3.1. Throughfall, soil temperature and moisture

The rain shelters were in use over 92 d for the summer TFE in 2014, and over 104 d for the spring TFE in 2015. At the onset of TFE, the soil moisture did not differ between the control and the TFE treatments (Fig. 1c; $P > 0.05$). The soil moisture was reduced significantly by $23 \pm 2\%$ (mean \pm s.e.m.) and $18 \pm 5\%$ during the summer and spring TFE periods, respectively (Fig. 1c; $P < 0.05$). During the summer TFE, the soil moisture was 0.29 ± 0.01 and $0.23 \pm 0.03 \text{ m}^3 \text{ m}^{-3}$ for the control and TFE treatments, respectively (Fig. 1c). Following the removal of the rain shelters, the soil moisture was restored at a slow rate due to limited precipitation in autumn of 2014 (Fig. 1a,c).

During the spring TFE, the soil moisture was 0.30 ± 0.01 and $0.24 \pm 0.03 \text{ m}^3 \text{ m}^{-3}$ in the control and TFE treatments, respectively (Fig. 1c). Following the removal of the rain shelters, the soil moisture quickly returned to the control treatment level due to heavy summer precipitation in 2015 (Fig. 1a,c). The soil temperature was similar, with 17.01 ± 0.07 and 17.10 ± 0.09 °C in the TFE and control treatments throughout the entire study period, respectively (Fig. 1b; $P > 0.05$).

3.2. Responses of soil respiration to throughfall exclusion

Prior to the TFE in 2014 and 2015, R_s did not differ between plots assigned to the TFE and the control treatments (Fig. 1d). The R_s was significantly decreased in the summer and the spring TFE in 2014 and 2015 (Fig. 1d). The R_s remained significantly lower in the TFE plots than in the control, once the rain shelters were removed in 2014, and remained lower until November 2014. However, we observed a slight R_s recovery following the removal of the rain shelters in 2015. From May to December 2014, $9.31 \pm 0.73 \text{ t C ha}^{-1}$ were released in the control, whereas only $6.67 \pm 0.50 \text{ t C ha}^{-1}$ were released in the TFE treatment (Table 2). This resulted in a difference of ca. 2.62 t C ha^{-1} , where 65% of this difference (or approximately 1.69 t C ha^{-1}) resulted from reduced R_s during the exclusion period, while the remaining portion was contributed during the post-treatment period. In 2015, $10.98 \pm 1.09 \text{ t C ha}^{-1}$ and $7.52 \pm 0.63 \text{ t C ha}^{-1}$ were released under the control and the TFE treatments, respectively (Table 2). This resulted in a reduction of ca. 3.46 t C ha^{-1} , where 38% of the reduction was from the decreased R_s during the exclusion period itself, whereas the remaining portion was from the post-treatment period.

Soil temperature accounted for 88% of the variation in R_s in the

Table 2

Cumulative C flux emissions (mean \pm s.e.m., $n = 3$) from the control and the throughfall exclusion (TFE) treatments in the untrenched and trenched subplots.

Year	Period	Duration (days)	Cumulative C flux (t C ha ⁻¹)			
			untrenched		trenched	
			Control	TFE	Control	TFE
2014	Pre-treatment	35	1.17 \pm 0.08a	1.13 \pm 0.07a	0.82 \pm 0.04a	0.80 \pm 0.07a
	TFE	92	4.98 \pm 0.29a	3.29 \pm 0.21b	3.64 \pm 0.22a	2.58 \pm 0.29b
	Post-treatment	110	3.16 \pm 0.36a	2.25 \pm 0.22b	2.04 \pm 0.13a	2.03 \pm 0.26a
	Total	237	9.31 \pm 0.73a	6.67 \pm 0.50b	6.50 \pm 0.39a	5.41 \pm 0.62a
2015	Pre-treatment	74	0.99 \pm 0.13a	0.90 \pm 0.09a	0.67 \pm 0.07a	0.82 \pm 0.11a
	TFE	104	3.23 \pm 0.41a	1.90 \pm 0.16b	2.11 \pm 0.21a	1.69 \pm 0.19a
	Post-treatment	187	6.76 \pm 0.55a	4.73 \pm 0.37b	4.34 \pm 0.36a	4.11 \pm 0.42a
	Total	365	10.98 \pm 1.09a	7.52 \pm 0.63b	7.12 \pm 0.63a	6.62 \pm 0.71a

Different letter represent significant differences ($P < 0.05$) between control and TFE treatment in the untrenched and trenched subplots.

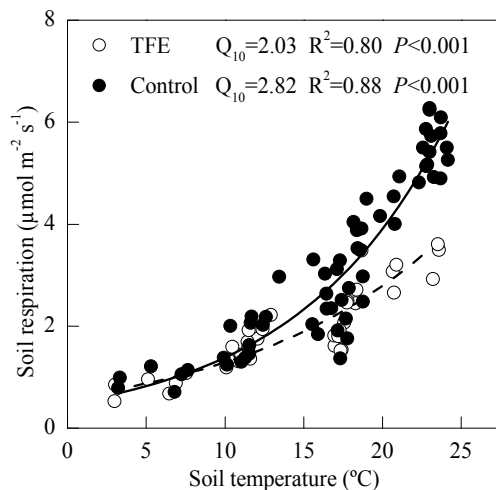


Fig. 2. Exponential relationship between soil respiration and soil temperature in the TFE plots and control plots during rewetting in 2014 and 2015.

control plots, with a Q_{10} of 2.82, indicated a strong temperature sensitivity of soil respiration (Fig. 2). Considering all measurements in R_s from the TFE plots during rewetting in 2014 and 2015, the soil temperature accounted for 83% of the variation in R_s . The Q_{10} of 2.03 in the TFE plots was significantly lower than that of the control plots.

3.3. Soil respiration partitioning

The effects of the droughts on R_a and R_h differed, where R_h was reduced by $18 \pm 5\%$ and $6 \pm 2\%$, and R_a was reduced by $50 \pm 10\%$ and $75 \pm 6\%$ in 2014 and 2015, respectively (Table 2; Fig. 3a and b). During the entire experimental period, R_a was on average $1.05 \pm 0.13 \mu\text{mol m}^{-2} \text{s}^{-1}$ in the control, and $0.36 \pm 0.03 \mu\text{mol m}^{-2} \text{s}^{-1}$ in the TFE plots (Fig. 3a,b), i.e., a reduction of $65 \pm 7\%$; R_h was on average $2.17 \pm 0.16 \mu\text{mol m}^{-2} \text{s}^{-1}$ in the control, and

$1.92 \pm 0.21 \mu\text{mol m}^{-2} \text{s}^{-1}$ in the TFE plots, i.e., a reduction of $12 \pm 3\%$. Thus, the relative contribution of R_a to R_s was significantly decreased from $33 \pm 1\%$ in the control treatments, to $16 \pm 3\%$ in the TFE treatments.

3.4. Microbial biomass C and fine root biomass

At the onset of the TFE experiment, the MBC did not differ between the control and the TFE plots (37.2 g m^{-2} versus 38.6 g m^{-2} ; Fig. 4a). Toward the end of the summer TFE, the MBC was significantly lower in the TFE than in the control plots, with $29.9 \pm 1.3 \text{ g m}^{-2}$ compared with $40.7 \pm 2.3 \text{ g m}^{-2}$. The MBC did not differ significantly between the TFE and the control treatments, which was measured two months following rewetting. Similarly, toward the end of the spring TFE, the MBC was significantly lower in the TFE plots ($34.2 \pm 1.6 \text{ g m}^{-2}$) than in the control plots ($47.4 \pm 2.7 \text{ g m}^{-2}$). Following rewetting, there were no significant differences in the MBC between the control and the TFE plots (Fig. 4a).

At the onset of the TFE experiment, the FRB did not differ between the TFE and the control treatments (170.7 g m^{-2} versus 164.4 g m^{-2} ; Fig. 4b). Toward the end of summer TFE, the FRB decreased by $57 \pm 2\%$ in the TFE plots compared with the control treatments. After rewetting, the difference in FRB between the control and the TFE plots gradually decreased over time. Similarly, at the end of the spring TFE, the FRB decreased by $47 \pm 2\%$ in the TFE compared with the control treatments. The FRB from the TFE treatments decreased by $19 \pm 4\%$ compared with the control two months following rewetting; however, the difference was statistically insignificant ($P = 0.321$).

4. Discussion

4.1. Effects of throughfall exclusion on soil respiration

As we hypothesized, our experiment revealed that prolonged droughts during the roof-closure decreased R_s and Q_{10} in R_s in the throughfall exclusion plots, compared with those in the control plots.

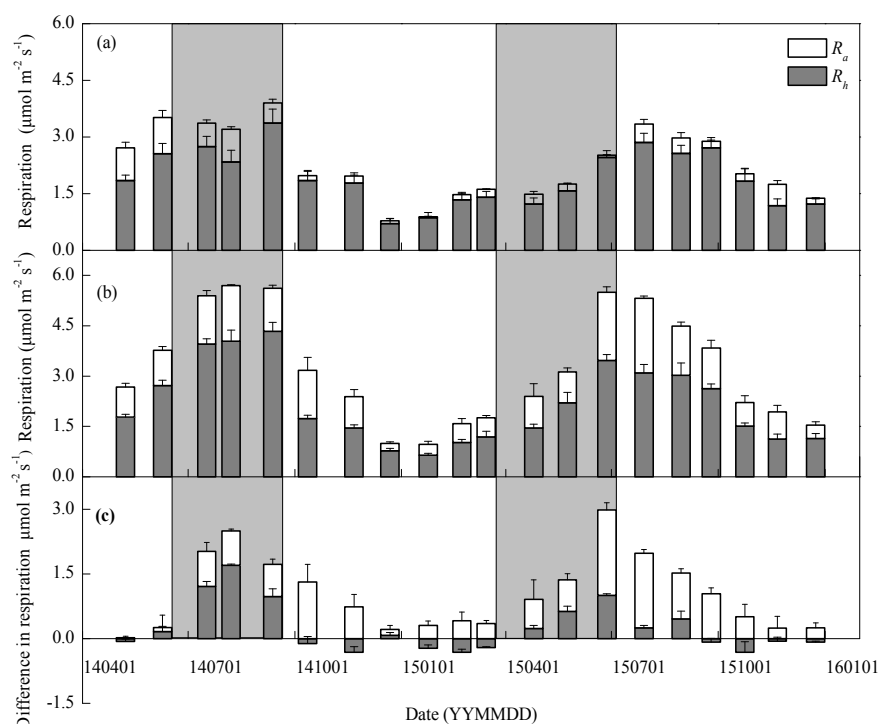


Fig. 3. Seasonal variations of autotrophic and heterotrophic soil respiration (R_a and R_h , respectively) in (a) the throughfall exclusion (TFE) treatments, (b) the control treatments and (c) the differences in R_a and R_h between the control and TFE treatments from May, 2014 to December, 2015.

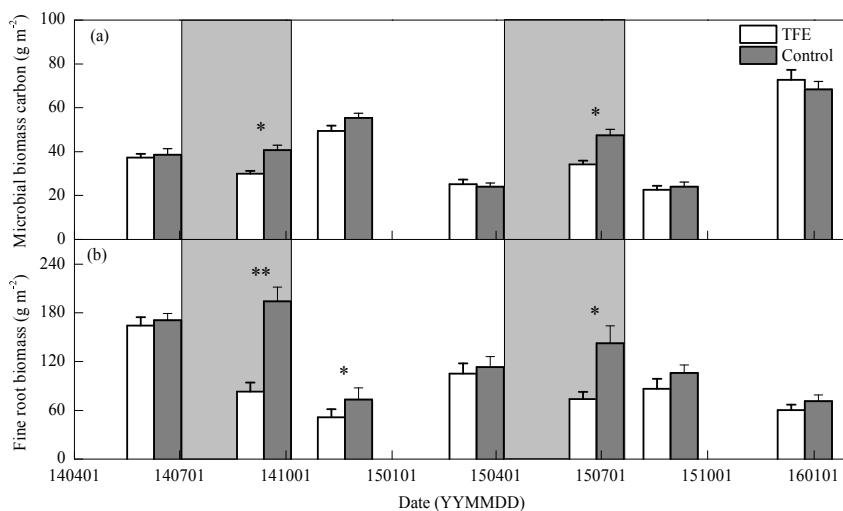


Fig. 4. Microbial biomass C (a) and fine root biomass (b) at 0–10 cm mineral soil depth in the control and TFE treatments prior to, during, and following rain shelter roof-closure, in 2014 and 2015. Shaded areas indicate periods when the rain shelter roofs were installed. Error bars represent the standard error of the means ($n = 3$). * = $P < 0.05$, ** = $P < 0.01$.

Our results agreed with those of [Muhr and Borken \(2009\)](#), who found that reduced matric potential in the organic layer and top mineral horizon due to throughfall exclusion, decreased the R_s in a mountainous Norway spruce forest in Southern Germany. Similar decreases in R_s were also observed under throughfall exclusion conditions in a temperate spruce forest and an Eastern Amazonian rainforest ([Sotta et al., 2007](#); [Schindlbacher et al., 2012](#)). Similarly, the reduced temperature sensitivity of R_s under throughfall reduction was also previously observed ([Borken et al., 2006](#); [Jiang et al., 2013](#)). In our study, the soil moisture decreased by approximately 23% and 18% toward the end of the TFE in 2014 and 2015, respectively. Because both the TFE and control plots experienced similar temperature regime in our study, we, therefore, ascribe this reduction in R_s to the drying out of the soil organic layer during the exclusion period ([Borken et al., 2006](#); [Schindlbacher et al., 2012](#)). Our result of a significant reduction of microbial biomass carbon in response to drought is consistent with the understanding that decreased soil water availability reduces diffusivity and the supply of substrates for microbes; thereby diminishing microbial activity ([Schimel et al., 2007](#); [Talmon et al., 2011](#); [Wang et al., 2014](#)). In the meantime, fine root biomass was significantly reduced by throughfall exclusion ([Fig. 4b](#)). Therefore, the negative effects of extended droughts on R_s we observed are attributable to the reduction of both soil microbes and fine root activity.

4.2. Effects of rewetting on soil respiration

We found an overall weak response in R_s to rewetting, which is consistent with several previous studies in natural environments ([Borken and Matzner, 2009](#); [Schindlbacher et al., 2012](#); [Hagedorn and Joos, 2014](#)). These authors attributed the slow recovery in R_s following rewetting, to hydrophobicity, whereby the complete drying out of the organic layer induced water flow along preferential paths that bypassed hydrophobic surfaces ([Bogner et al., 2010](#)), thus producing weak R_s responses after rewetting. Others, however, have observed that the rewetting may lead to a pulse in CO_2 releases, which could even exceed pre-drought levels ([Birch, 1958](#); [Borken et al., 2003](#)). This likely resulted from accumulated microbial and plant necromass, lysis of living microbial cells, the release of compatible solutes, and exposure of previously protected organic matter ([Borken and Matzner, 2009](#)). It is possible that “Birch effect” is contingent on the extent of soil drying. Alternatively, due to our monthly monitoring frequency in R_s , we might have omitted a very short-lived mineralization flush ([Borken and Matzner, 2009](#); [Hagedorn and Joos, 2014](#)).

Our study revealed that 65% and 38% of the total reduction in R_s occurred during the rain shelter-closure periods in 2014 and 2015, respectively, while the remaining reduction resulted from continuously

decreased R_s following the removal the rain shelters. A similar pattern was reported by [Muhr and Borken \(2009\)](#). In our two year experimental manipulation, R_s in the TFE plots did not return to the levels of the control plots until three to four months following the removal of the rain shelters. The lag in R_s recovery in 2014 is attributable to the fact that the soil moisture continuously declined due to the low autumnal rainfall. However, we observed a continuous decline in R_s following rewetting in 2015, although the soil moisture rapidly returned to control plot levels due to sufficient rainfall. The consistently low R_s following rewetting could have resulted from more severe damages to the metabolic capacity of the fine roots than microbial activity since we found that the microbial biomass C recovered more quickly following rewetting. The more rapid recovery of microbial activity might have been attributable to soil microbial strategies, such as changes in community composition, as well as shifts within taxa associated with drying-rewetting cycles ([Wang et al., 2013](#); [Evans and Wallenstein, 2014](#)).

4.3. Responses of different respiration components on throughfall exclusion

Contrary to our hypothesis, extended drought decreased R_a more significantly than R_h . Our results were supported by a more pronounced negative effect in fine root biomass than on microbial biomass C. Our findings were consistent with those of [Hinko-Najera et al. \(2015\)](#) and [Kopittke et al. \(2014\)](#), who reported that R_a was more suppressed than R_h during prolonged drought in a Dutch *Calluna vulgaris* heathland, and in a dry temperate broadleaved evergreen Eucalypt forest. However, these results contrasted with those of other researchers ([Borken et al., 2006](#); [Muhr and Borken, 2009](#); [Wang et al., 2014](#)). Different responses in R_a and R_h to drought might result from differences in root phenology ([Carbone et al., 2011](#)), and the internal plant C allocation, by shifting more C to growth than R_a ([Doughty et al., 2015](#); [Hinko-Najera et al., 2015](#)). On the one hand, the temporal variations in R_a were driven by phenological changes in root biomass ([Fig. 4b](#)). In addition, the availability of substrate and the allocation of recent photosynthates to aboveground and belowground vegetation parts will determine the rate of the autotrophic soil respiration. In our drought experiment, fine root activity was severely weakened due to long-term drought, and R_a decreased significantly. Therefore, the contribution of R_a to total soil respiration significantly decreased, suggested that the more pronounced negative effect in R_a than R_h .

The trenching method was most commonly applied in forest ecosystems ([Chen et al., 2011](#)), and may result in higher R_h/R_s ratios because disturbance and residual roots left by trenching may stimulate microbial respiration ([Kuzyakov, 2006](#); [Sayer and Tanner, 2010](#)). However, the increase in soil respiration stimulated by trenching tends

to transient and short-lived (Subke et al., 2006). Our data are in support with trenching stimulation is short lived as we found no statistical difference in R_s contributions to total R_s between 2014 and 2015 ($P = 0.341$, TFE; $P = 0.114$, Control), which suggested the severed roots in the trenched subplot contributed little to the total soil respiration six month after root removal. Moreover, root removal could increase soil moisture due to the lack of evapotranspiration from live vegetation. Therefore, we examined soil moisture in the trenched and untrenched subplots. The difference, however, was rather small (Fig. 1c, $P = 0.468$, TFE; $P = 0.223$, Control), and the effect of such a difference in soil moisture would have no effect on soil respiration. Taking together, our estimates of R_h and R_a appear to be reasonable and available. In this study, we sampled fine roots at 0–10 cm soil depth. We note that, although fine root biomass at 0–10 cm represents a large portion of the entire soil profile (Feng et al., 2018), the response of fine roots to drought could differ with soil depth. Future efforts are needed to examine the responses of fine roots in deeper soil layers to drought.

5. Conclusion

In this subtropical forest in the Southeast China, prolonged droughts strongly reduced R_s . Moreover, we observed that R_s did not recover until several months following rewetting, potentially due to serious damages to fine root activity. In addition, the negative effects of droughts were more pronounced for R_a than R_h . Our findings highlight the need to account for asymmetric responses to droughts between R_a and R_h when predicting the responses of ecosystem C balances in future drought events.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.soilbio.2018.04.029>.

References

- Beier, C., Beierkuhnlein, C., Wohlgemuth, T., Penuelas, J., Emmett, B., Körner, C., Boeck, H.D., Christensen, J.H., Leuzinger, S., Janssens, I.A., 2012. Precipitation manipulation experiments - challenges and recommendations for the future. *Ecology Letters* 15, 899–911.
- Birch, H.F., 1958. The effect of soil drying on humus decomposition and nitrogen availability. *Plant and Soil* 10, 9–31.
- Bogner, C., Gaul, D., Kolb, A., Schmiedinger, I., Huwe, B., 2010. Investigating flow mechanisms in a forest soil by mixed-effects modelling. *European Journal of Soil Science* 61, 1079–1090.
- Bond-Lamberty, B., Thomson, A., 2010. Temperature-associated increases in the global soil respiration record. *Nature* 464, 579–582.
- Borken, W., Davidson, E.A., Savage, K., Gaudinski, J., Trumbore, S.E., 2003. Drying and wetting effects on carbon dioxide release from organic horizons. *Soil Science Society of America Journal* 67, 1888–1896.
- Borken, W., Matzner, E., 2009. Reappraisal of drying and wetting effects on C and N mineralization and fluxes in soils. *Global Change Biology* 15, 808–824.
- Borken, W., Savage, K., Davidson, E.A., Trumbore, S.E., 2006. Effects of experimental drought on soil respiration and radiocarbon efflux from a temperate forest soil. *Global Change Biology* 12, 177–193.
- Brassard, B.W., Chen, H.Y.H., Cavard, X., Laganière, J., Reich, P.B., Bergeron, Y., Paré, D., Yuan, Z., Chen, H., 2013. Tree species diversity increases fine root productivity through increased soil volume filling. *Journal of Ecology* 101, 210–219.
- Bu, X., Ruan, H., Wang, L., Ma, W., Ding, J., Yu, X., 2012. Soil organic matter in density fractions as related to vegetation changes along an altitude gradient in the Wuyi Mountains, southeastern China. *Applied Soil Ecology* 52, 42–47.
- Carbone, M.S., Still, C.J., Ambrose, A.R., Dawson, T.E., Williams, A.P., Boot, C.M., Schaeffer, S.M., Schimel, J.P., 2011. Seasonal and episodic moisture controls on plant and microbial contributions to soil respiration. *Oecologia* 167, 265–278.
- Chen, G.S., Yang, Y.S., Guo, J.F., Xie, J.S., Yang, Z.J., 2011. Relationships between carbon allocation and partitioning of soil respiration across world mature forests. *Plant Ecology* 212, 195–206.
- Cleveland, C.C., Wieder, W.R., Reed, S.C., Townsend, A.R., 2010. Experimental drought in a tropical rain forest increases soil carbon dioxide losses to the atmosphere. *Ecology* 91, 2313–2323.
- Dai, A., 2011. Drought under global warming: a review. *Wiley Interdisciplinary Reviews Climate Change* 2, 45–65.
- Doughty, C.E., Metcalfe, D.B., Girardin, C.A., Amezquita, F.F., Cabrera, D.G., Huasco, W.H., Silva-Espejo, J.E., Araujo-Murakami, A., da Costa, M.C., Rocha, W., Feldpausch, T.R., Mendoza, A.L., da Costa, A.C., Meir, P., Phillips, O.L., Malhi, Y., 2015. Drought impact on forest carbon dynamics and fluxes in Amazonia. *Nature* 519, 78–82.
- Evans, S.E., Wallenstein, M.D., 2014. Climate change alters ecological strategies of soil bacteria. *Ecology Letters* 17, 155–164.
- Feng, C., Wang, Z., Zhu, Q., Fu, S., Chen, H.Y.H., 2018. Rapid increases in fine root biomass and production following cessation of anthropogenic disturbances in degraded forests. *Land Degradation & Development*. <http://dx.doi.org/10.1002/ldr.2878>.
- Gimbel, K.F., Felsmann, K., Baudis, M., Puhlmann, H., Gessler, A., Bruehlheide, H., Kayler, Z., Ellerbrock, R.H., Ulrich, A., Welk, E., 2015. Drought in forest understorey ecosystems - a novel rainfall reduction experiment. *Biogeosciences* 12, 14319–14358.
- Hagedorn, F., Joos, O., 2014. Experimental summer drought reduces soil CO₂ effluxes and DOC leaching in Swiss grassland soils along an elevational gradient. *Biogeochemistry* 117, 395–412.
- Hanson, P.J., Edwards, N.T., Garten, C.T., Andrews, J.A., 2000. Separating root and soil microbial contributions to soil respiration: a review of methods and observations. *Biogeochemistry* 48, 115–146.
- Hinko-Najera, N., Fest, B., Livesley, S.J., Arndt, S.K., 2015. Reduced throughfall decreases autotrophic respiration, but not heterotrophic respiration in a dry temperate broad-leaved evergreen forest. *Agricultural and Forest Meteorology* 200, 66–77.
- IPCC, 2013. Climate change 2013: the physical science basis. In: Stocker, T.F., Qin, D., Plattner, G.K., Tignor, M., Allen, S.K., Boschung, J., Nauels, A., Xia, Y., Bex, V., Midgley, P.M. (Eds.), Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge.
- Jiang, H., Deng, Q., Zhou, G., Hui, D., Zhang, D., Liu, S., Chu, G., Li, J., 2013. Responses of soil respiration and its temperature/moisture sensitivity to precipitation in three subtropical forests in southern China. *Biogeosciences* 10, 3963–3982.
- Joergensen, R.G., 1996. The fumigation-extraction method to estimate soil microbial biomass: calibration of the k_{EC} value. *Soil Biology and Biochemistry* 28, 33–37.
- Kopittke, G.R., Tietema, A., van Loon, E.E., Asscheman, D., 2014. Fourteen annually repeated droughts suppressed autotrophic soil respiration and resulted in an ecosystem change. *Ecosystems* 17, 242–257.
- Kuzyakov, Y., 2006. Sources of CO₂ efflux from soil and review of partitioning methods. *Soil Biology and Biochemistry* 38, 425–448.
- Kuzyakov, Y., 2010. Priming effects: interactions between living and dead organic matter. *Soil Biology and Biochemistry* 42, 1363–1371.
- Laganière, J., Paré, D., Bergeron, Y., Chen, H.Y.H., 2012. The effect of boreal forest composition on soil respiration is mediated through variations in soil temperature and C quality. *Soil Biology and Biochemistry* 53, 18–27.
- Lellei-Kovács, E., Botta-Dukát, Z., Dato, G.D., Estiarte, M., Guidolotti, G., Kopittke, G.R., Kovács-Láng, E., Kröel-Dulay, G., Larsen, K.S., Peñuelas, J., 2016. Temperature dependence of soil respiration modulated by thresholds in soil water availability across European shrubland ecosystems. *Ecosystems* 19, 1–18.
- Li, D., Zhou, X., Wu, L., Zhou, J., Luo, Y., 2013. Contrasting responses of heterotrophic and autotrophic respiration to experimental warming in a winter annual-dominated prairie. *Global Change Biology* 19, 3553–3564.
- Liu, Y., Liu, S., Wan, S., Wang, J., Luan, J., Wang, H., 2016. Differential responses of soil respiration to soil warming and experimental throughfall reduction in a transitional oak forest in central China. *Agricultural and Forest Meteorology* 226–227, 186–198.
- Lloyd, J., Taylor, J.A., 1994. On the temperature dependence of soil respiration. *Functional Ecology* 8, 315–323.
- Lu, M., Zhou, X., Yang, Q., Li, H., Luo, Y., Fang, C., Chen, J., Yang, X., Li, B., 2013. Responses of ecosystem carbon cycle to experimental warming: a meta-analysis. *Ecology* 94, 726–738.
- Manzoni, S., Schimel, J.P., Porporato, A., 2012. Responses of soil microbial communities to water stress: results from a meta-analysis. *Ecology* 93, 930–938.
- Mi, N., Yu, G., Wen, W., Sun, X., Wang, S., Zhang, L., Song, X., 2009. Use of ecosystem flux data and a simulation model to examine seasonal drought effects on a subtropical coniferous forest. *Asia-Pacific Journal of Atmospheric Sciences* 45, 207–220.
- Muhr, J., Borken, W., 2009. Delayed recovery of soil respiration after wetting of dry soil further reduces C losses from a Norway spruce forest soil. *Journal of Geophysical Research Atmospheres* 114, 1088.
- Muhr, J., Höhle, J., Otieno, D.O., Borken, W., 2011. Manipulative lowering of the water table during summer does not affect CO₂ emissions and uptake in a fen in Germany. *Ecological Applications* 21, 391–401.
- Noh, N.J., Kuribayashi, M., Saitoh, T.M., Nakaji, T., Nakamura, M., Hiura, T., Muraoka, H., 2015. Responses of soil, heterotrophic, and autotrophic respiration to experimental open-field soil warming in a cool-temperate deciduous forest. *Ecosystems* 19, 504–520.

- Noh, N.J., Kuribayashi, M., Saitoh, T.M., Muraoka, H., 2017. Different responses of soil, heterotrophic and autotrophic respirations to a 4-year soil warming experiment in a cool-temperate deciduous broadleaved forest in central Japan. *Agricultural and Forest Meteorology* 247, 560–570.
- Sanaullah, M., Rumpel, C., Charrier, X., Chabbi, A., 2012. How does drought stress influence the decomposition of plant litter with contrasting quality in a grassland ecosystem? *Plant and Soil* 352, 277–288.
- Sayer, E.J., Tanner, E.V.J., 2010. A new approach to trenching experiments for measuring root–rhizosphere respiration in a lowland tropical forest. *Soil Biology and Biochemistry* 42, 347–352.
- Schimel, J., Balser, T.C., Wallenstein, M., 2007. Microbial stress-response physiology and its implications for ecosystem function. *Ecology* 88, 1386–1394.
- Schindlbacher, A., Wunderlich, S., Borken, W., Kitzler, B., Zechmeister-Boltenstern, S., Jandl, R., 2012. Soil respiration under climate change: prolonged summer drought offsets soil warming effects. *Global Change Biology* 18, 2270–2279.
- Schindlbacher, A., Zechmeister-Boltenstern, S., Jandl, R., 2009. Carbon losses due to soil warming: do autotrophic and heterotrophic soil respiration respond equally? *Global Change Biology* 15, 901–913.
- Schlesinger, W.H., Andrews, J.A., 2000. Soil respiration and the global carbon cycle. *Biogeochemistry* 48, 7–20.
- Scott-Denton, L.E., Rosenstiel, T.N., Monson, R.K., 2006. Differential controls by climate and substrate over the heterotrophic and rhizospheric components of soil respiration. *Global Change Biology* 12, 205–216.
- Sherman, C., Sternberg, M., Steinberger, Y., 2012. Effects of climate change on soil respiration and carbon processing in Mediterranean and semi-arid regions: an experimental approach. *European Journal of Soil Biology* 52, 48–58.
- Sotta, E.D., Veldkamp, E., Schwendenmann, L., Guimarães, B.R., Paixão, R.K., Ruivo, M.d.L.P., Lola da Costa, A.C., Meir, P., 2007. Effects of an induced drought on soil carbon dioxide (CO₂) efflux and soil CO₂ production in an Eastern Amazonian rainforest. *Brazilian Global Change Biology* 13, 2218–2229.
- Subke, J.A., Inghima, I., Francesca Cotrufo, M., 2006. Trends and methodological impacts in soil CO₂ efflux partitioning: a metaanalytical review. *Global Change Biology* 12, 921–943.
- Suseela, V., Dukes, J.S., 2013. The responses of soil and rhizosphere respiration to simulated climatic changes vary by season. *Ecology* 94, 403–413.
- Talmon, Y., Sternberg, M., Grünzweig, J.M., 2011. Impact of rainfall manipulations and biotic controls on soil respiration in Mediterranean and desert ecosystems along an aridity gradient. *Global Change Biology* 17, 1108–1118.
- Van Straaten, O., Veldkamp, E., Corre, M.D., 2011. Simulated drought reduces soil CO₂ efflux and production in a tropical forest in Sulawesi, Indonesia. *Ecosphere* 2, 218–227.
- Vance, E.D., Brookes, P.C., Jenkinson, D.S., 1987. An extraction method for measuring soil microbial biomass C. *Soil Biology and Biochemistry* 19, 703–707.
- Wang, Y., Hao, Y., Cui, X.Y., Zhao, H., Xu, C., Zhou, X., Xu, Z., 2014. Responses of soil respiration and its components to drought stress. *Journal of Soils and Sediments* 14, 99–109.
- Wang, Z., Sun, G., Luo, P., Mou, C., Wang, J., 2013. A study of soil-dynamics based on a simulated drought in an alpine meadow on the Tibetan Plateau. *Journal of Mountain Science* 10, 833–844.
- Wessel, W.W., Tietema, A., Beier, C., Emmett, B.A., Peñuelas, J., Riis-Nielsen, T., 2004. A qualitative ecosystem assessment for different shrublands in Western Europe under impact of climate change. *Ecosystems* 7, 662–671.
- Xu, X., Shi, Z., Li, D., Zhou, X., Sherry, R.A., Luo, Y., 2015. Plant community structure regulates responses of prairie soil respiration to decadal experimental warming. *Global Change Biology* 21, 3846–3853.
- Yu, G., Chen, Z., Piao, S., Peng, C., Ciais, P., Wang, Q., Li, X., Zhu, X., 2014. High carbon dioxide uptake by subtropical forest ecosystems in the East Asian monsoon region. *Proceedings of the National Academy of Sciences of the United States of America* 111, 4910–4915.
- Zhang, X., Zhang, Y., Sha, L., Wu, C., Tan, Z., Song, Q., Liu, Y., Dong, L., 2015. Effects of continuous drought stress on soil respiration in a tropical rainforest in southwest China. *Plant and Soil* 394, 343–353.